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Cardiovascular disease in Systemic Sclerosis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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CARDIOVASCULAR DISEASE IN SYSTEMIC SCLEROSIS

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ABSTRACT

Systemic Sclerosis (SSc) is an autoimmune rheumatic disease, characterized by fibrosis of the skin and internal organs, vasculopathy and the production of autoantibodies. Cardiac and pulmonary disease manifestations are major causes of morbidity and mortality. Microvascular manifestations such as digital ulcers and Raynaud's phenomenon are common, but whether macrovascular disease is also enhanced in SSc has not been clear. In a cross-sectional design we examined the heart, the macro- and microvasculature and how vascular disease manifestations correlated to each other, to SSc subsets and to biomarkers. Population based age- and gender-matched controls were used as comparators.

Carotid atherosclerosis, measured by ultrasound, and history of ischemic arterial events (IAE) were investigated in 111 SSc patients and 105 controls. Previous IAE were three times more common in the patients, especially ischemic heart disease (IHD) and ischemic peripheral vascular disease (IPVD). Measures of atherosclerosis, i.e. intima media thickness, plaque occurrence and ankle-brachial index, did not differ on a group level, but patients with anticentromere antibodies (ACA+) had more IAE and more plaques than ACA- patients and controls. Echocardiographic (echo) findings and their association with cardiac troponin I (cTnI), N-terminal pro brain natriuretic hormone (NT-proBNP) and Uric Acid (UA) were investigated. The patients had higher levels than controls of cTnI and NT-proBNP but not of UA. All three biomarkers were associated with echo abnormalities among the SSc patients. A low left ventricular ejection fraction (LVEF) and hypokinesia were more common in patients but associated with previous IHD. Patients had more valve regurgitations than controls and 15 patients, but no controls, had an elevated echo-estimated systolic pulmonary arterial pressure (ePAP). A standard 12-lead electrocardiogram (ECG) was performed on all, and Holter-ECG on a subgroup of participants. Conduction defects, especially left bundle branch block (LBBB) and ventricular extrasystoles were more common in the patient group. Finally, the nailfolds of 163 SSc patients were examined by widefield microscopy for signs of dilated capillaries, avascular areas and capillary density. SSc patients with a higher ePAP, ACA+, digital ulcers and IAE, especially IPVD, had a lower nailfold capillary density

These studies demonstrate that patients with SSc had more IAE than controls, and SSc patients with previous IAE also have a disturbed microcirculation in the nailfolds. It is a new observation that both macrovascular IAEs and accelerated atherosclerosis prevails in the ACA+ SSc subgroup. SSc patients had more valve regurgitations, higher ePAP and more left and right ventricular impairment on echo. These echo abnormalities were associated with higher levels of the cardiac biomarkers, NT-proBNP and cTnI. The latter is a new clinically useful finding.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following original papers and manuscripts, which will be referred to in the text by their Roman numerals.

- I. **Nordin A**, Jensen-Urstad K, Björnådal L, Pettersson S, Larsson A, Svenungsson E. Ischemic arterial events and atherosclerosis in patients with systemic sclerosis: a population-based case-control study. *Arthritis Research & Therapy* 2013, 15:R87
- II. **Nordin A**, Björnådal L, Larsson A, Svenungsson E, Jensen-Urstad K. Electrocardiogram in 110 patients with systemic sclerosis: a cross-sectional study with population-based controls. *Scand J Rheumatol* 2013; First article: 1–5
- III. **Nordin A**, Svenungsson E, Björnådal L, Larsson A, Jensen-Urstad K. Echocardiography and Troponin I in patients with systemic sclerosis (SSc) and matched population controls. Manuscript
- IV. **Nordin A**, Björnådal L, Roubi L, Jensen-Urstad K, Svenungsson E. Microcirculation and macrovascular disease in systemic sclerosis – a nailfold capillary study. Manuscript

CONTENTS

1	Systemic sclerosis	1
1.1	Introduction:	1
1.2	Classification Criteria:	1
1.3	Epidemiology.....	4
1.4	Pathogenesis	5
1.4.1	Fibrosis.....	5
1.4.2	Vasculopathy	6
1.4.3	Activation of immune system:.....	8
1.5	Clinical features	9
1.6	Treatment:	10
2	Cardiovascular disease and atherosclerosis.....	11
2.1	Atherosclerosis	11
2.2	Methods to measure atherosclerosis and endothelial dysfunction: .	12
2.3	Microcirculation:	13
2.4	Cardiac fibrosis:.....	15
2.5	Methods to study the heart:	16
2.6	Biomarkers for cardiac damage:	17
2.7	Other biomarkers	19
2.8	Traditional risk factors:	20
3	Cardiovascular disease and atherosclerosis in systemic sclerosis	21
3.1	The heart in systemic sclerosis:	21
3.2	Macrovascular disease and atherosclerosis in SSc	24

3.3	Microvascular disease in systemic sclerosis:	26
4	Aims:	28
5	Methods.....	29
5.1	Study populations	29
5.2	Definition of disease activity, severity, organ involvement and cardiovascular events:.....	31
5.3	Assessment of atherosclerosis, heart rhythm, heart function and microvascular structure:	34
6	Results:.....	40
7	Discussion:	44
8	Concluding remarks and future perspectives:	48
9	Acknowledgements.....	50
10	References.....	52

LIST OF ABBREVIATIONS

ACA	Anti-centromere antibody
ACE	Angiotensine converting enzyme
ACR	American College of Rheumatology
ANA	Antinuclear antibodies
ANP	Atrium natiuretic peptide
ARA	Anti Ribonuclein polymeras III antibodies
ARA	American Rheumatism Association
ATA	Antitopoisomeras I antibodies
BNP	Brain natiuretic peptide
CRP	C-reactive protein
cTn	Cardiac troponin
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
dcSSc	Diffuse cutaneous systemic sclerosis
EC	Endothelial cell
ECG	Electrocardiogram
ECM	Extracellular Matrix
EF	Ejection fraction
ePAP	Estimated pulmonary arterial pressure
EULAR	European League against Rheumatism
FMD	Flow mediated dilatation
HDL	High density lipoproteins

Hs	High sensitivity
HR	Hazard Ratio
IAE	Ischemic arterial event
ICAM	Intercellular adhesion molecule
ICVD	Ishemic cerebrovascular disease
IMT	Intima media tickness
IPVD	Ischemic pheripheral vascular disease
lcSSc	Limited cutaneous systemic sclerosis
LDL	Low density lipoprotein
LV	Left ventricle
MI	Myocardial infarction
MHz	Mega Hertz
NT-proBNP	N-terminal prohormone of brain natiuretic peptide
NO	Nitric oxide
OR	Odds ratio
PAH	Pulmonary arterial hypertension
PGI ₂	Prostaglandin 2
PH	Pulmonary hypertension
RP	Raynaud's Phenomenon
RV	Right ventricula
ROS	Reactive oxygen scavanger
SSc	Systemic sclerosis
SRC	Scleroderma renal crisis
UA	Uric acid

VEGF	Vascular endothelium growth factor
VCAM	Vascular cellular adhesion molecule

1 SYSTEMIC SCLEROSIS

1.1 INTRODUCTION:

Systemic sclerosis (SSc) is a relatively rare rheumatic disease affecting a diversity of organs (1). The first known patient case was described in 1753 when the Italian doctor Carlo Curtzio described a young female with hard, wooden like and cold skin, despite good pulses. The term scleroderma was introduced around 1850, but it was not until the beginning of the 1900th century that it became clear that many other organs than the skin are affected in this disease. Maurice Raynaud, who gave the cold induced colour-changing of the fingers the eponym “Raynaud’s phenomenon”, was the first to realize that there was an association between the cold, colour changing of the fingers and systemic sclerosis (2).

The combination of five different disease characteristics, namely calcinosis, Raynaud’s phenomenon, esophagus involvement, sclerodactyly and telangiectasia was introduced in 1964 as the CREST-syndrome, a sub type of scleroderma (3) to be distinguished from the more severe “progressive systemic sclerosis”. Although many have suggested abandoning these names because of imprecise and overlapping features (4, 5) the International Statistical Classification of Diseases and Health Problems, 10th Revision (ICD-10) still distinguishes between the terms CREST (M 34.1) and progressive systemic sclerosis (M34.0) (6)

1.2 CLASSIFICATION CRITERIA:

In 1980 the American Rheumatism Association (ARA, today called American College of Rheumatology; ACR) published preliminary classification criteria for SSc (7). Although preliminary, these criteria have been used in almost every clinical study since then. Their purpose is to correctly select patients with established SSc for scientific studies and to distinguish the entity from other more localized scleroderma-like disorders:

Table 1. The 1980 ARA major and minor criteria for the diagnosis of SSc.

Major criterion:	Proximal scleroderma: Typical sclerodermatous skin changes: tightness, thickening, and non-pitting induration, excluding the localized forms of scleroderma, proximal to metacarpophalangeal or metatarsophalangeal joints; affecting other parts of the extremities, face, neck, or trunk (thorax or abdomen); usually bilateral, symmetrical, and almost always <i>including</i> sclerodactyly
Minor criteria:	1. Sclerodactyly: above-indicated changes limited to fingers and toes
	2. Digital pitting scars or loss of substance from the finger pad: depressed areas at tips of digits or loss of digital pad tissue as a result of digital ischemia rather than trauma or exogenous causes
	3. Bibasilar pulmonary fibrosis: bilateral reticular pattern of linear or lineo-nodular densities which are most pronounced in basilar portions of the lungs on standard chest roentgenogram; may assume appearance of diffuse mottling or “honeycomb lung”, and should not be attributable to primary lung disease.

Patients with the major criterion or 2 minor criteria are classified as having Systemic sclerosis

A further subgrouping of the patients was suggested in 1988 by LeRoy et al, dividing the patients, depending on how widespread the skin involvement is, into diffuse cutaneous SSc (dcSSc-skin sclerosis on trunk, thighs, upper arms, abdomen), limited cutaneous SSc (lcSSc- skin sclerosis confined to arms and legs below elbows and knees and/or skin sclerosis of the face) and limited Systemic sclerosis (lSSc- a patient group which lacks the characteristic fibrotic skin, but otherwise has the characteristics of SSc) (4).

The preliminary 1980 ARA criteria have a good specificity for recognising established SSc patients. But, the last 20 years many efforts have been made to identify the patients earlier, before irreversible organ damages occurs. Many studies have demonstrated that antinuclear antibodies (ANA), in particular some sub-specificities (anticentromere, anti-topoisomerase I/anti-Scl-70, anti-RNA polymerase III), and deranged nail fold capillary loops had a high predictive value for the development of SSc in the future (8-10) These observations have lead to the development of the new ACR/EULAR 2013 classification criteria for Systemic sclerosis (11).

Table 2. The American College of Rheumatology/European League Against Rheumatism criteria for the classification of systemic sclerosis (SSc)*

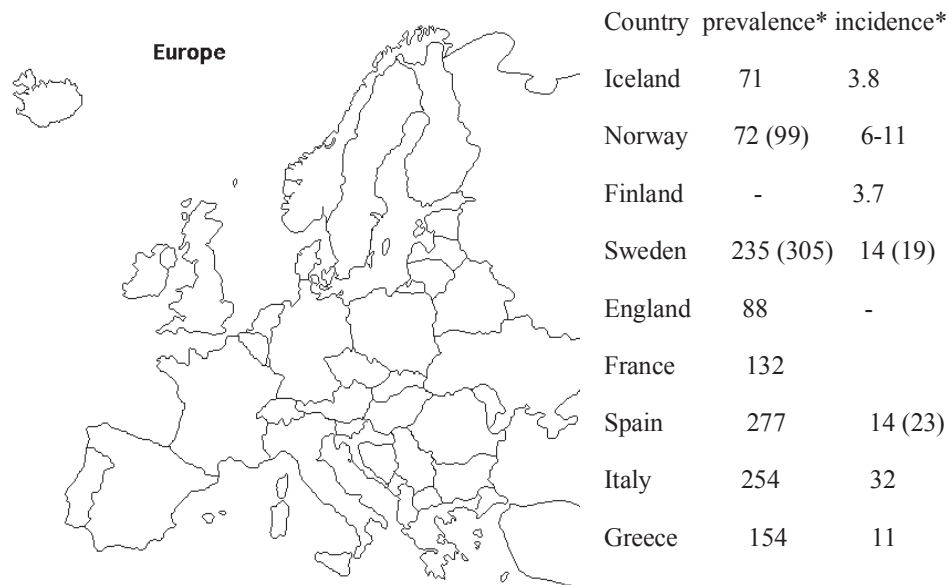
Item	Sub-item(s)	Weight/score†
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (sufficient criterion)	-	9
Skin thickening of the fingers (only count the higher score)	Puffy fingers	2
	Sclerodactyly of the fingers (distal to the MCP joints but proximal to the PIP joints)	4
Fingertip lesions (only count the higher score)	Digital tip ulcers	2
	Fingertip pitting scars	3
Telangiectasia	-	2
Abnormal nailfold capillaries	-	2
Pulmonary arterial hypertension and/or interstitial lung disease (maximum score is 2)	Pulmonary arterial hypertension	2
	Interstitial lung disease	2
Raynaud's phenomenon	-	3
SSc-related autoantibodies (anticentromere, anti-topoisomerase I/anti-Scl-70, anti-RNA polymerase III) (maximum score is 3)	Anticentromere antibodies (ACA)	3
	Anti-topoisomerase I (ATA)	3
	Anti-RNA polymerase III (ARA)	3

* These criteria are applicable to any patient considered for inclusion in an SSc study. The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestations (e.g., nephrogenic sclerosing fibrosis, generalized morphea, eosinophilic fasciitis, scleredema diabeticorum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy). † The total score is determined by adding the maximum weight (score) in each category. **Patients with a total score of ≥ 9 are classified as having definite SSc.**

1.3 EPIDEMIOLOGY

The incidence and prevalence of systemic sclerosis is hard to examine, especially because of the long delay between the onset of the first symptom to diagnosis, the overlap between SSc and other rheumatic disease and “the scleroderma mimics”. Two recent reviews of epidemiological studies show a large difference between countries, with prevalence in general between 50-300/ million inhabitants and incidence 4-22/million inhabitants. The highest incidence and prevalence in the USA and Australia, and the lowest in northern Europe and Asia (12, 13). Some have also described a latitude dependent distribution of the disease with a higher prevalence in the south regions, especially in Europe (13). A new study from Sweden opposed this theory by finding a relatively high prevalence of SSc patients, especially when using the new classification criteria (14). The observations that SSc is clustered in some areas have given rise to the suspicion that environmental exposures such as silica dust or organic solvents are important for the pathogenesis of SSc (15). The disease affects more females than males, with ratios varying between 5-8:1.

Figure 1: Prevalence and incidence of Systemic sclerosis in Europe.



References: (14, 16-23)

1.4 PATHOGENESIS

There are three main features involved in the pathogenesis of systemic sclerosis:

1.4.1 Fibrosis

The connective tissue offers a structural network to support organ function. The most common cell found in the connective tissue is the fibroblast, the major contributor to connective tissue homeostasis. The fibroblasts are highly active cells that regulate extracellular matrix (ECM) turnover through the production of both matrix metalloproteinases (MMPs), which degrades EMC, and tissue inhibitors of metalloproteinases (TIMPs), which inhibits the MMPs (24).

Serum levels and skin biopsy expression of TIMPs are higher in SSc-patients as compared with controls, suggesting an imbalance in ECM turnover (25, 26).

When a tissue is damaged, fibroblast can transform into myofibroblasts. These cells have a structure “somewhere between a fibroblast and a smooth muscle cell” and they are identified by their profound endoplasmatic reticula in combination with contractile stress filaments expressing alpha-smooth muscle actin. The origin of the myofibroblasts is not only the fibroblast; they can also develop from epithelial-, endothelial- or smooth muscle-cells. Myofibroblasts produce many ECM proteins, the most abundant are collagen type I and type III. They are important for repair of damaged tissues. When the repair process is completed the myofibroblasts disappear from the wound sites through apoptosis, deactivated or cleared by other cells. They leave a hypocellular scar tissue (27).

In fibrotic tissue, such as the SSc skin or the lung in SSc, the myofibroblast persist, which seems to depend on defective apoptosis and an enhanced sensibility to growth factors and pro-fibrotic stimuli (28). The SSc-fibroblasts

seem to produce excess ECM proteins, such as collagen, even in the absence of extrinsic stimuli, suggesting an autocrine activation (29).

The first event, initiating the transformation of fibroblasts to myofibroblasts in SSc, is likely activation of endothelial cells and platelets caused by infection, ischemia-reperfusion, toxins or autoantibodies resulting in tissue infiltration of inflammatory cells and secretion of different cytokines and growth factors. Transforming growth factor- β (TGF- β) has been considered the main regulator of fibrogenesis. It is mainly produced by macrophages but also by other cells, including endothelial cells. Both in vivo and in vitro studies have highlighted the importance of TGF- β in SSc (30).

There are also other cytokines, which play a crucial role in fibrogenesis such as connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), interleukin (IL)-1 β , IL-4, IL-6, IL-13, IL-17A, and type I interferon (IFN) (31).

1.4.2 Vasculopathy

Vascular abnormalities are a major component of SSc, but little is known about the events or mechanisms that initiate the vascular injury. The earliest clinical symptom of SSc, Raynaud's phenomenon, relates to disturbances in the peripheral vascular system that may precede other manifestations by years. The initial events leading to vascular alterations are thought to, in similarity to the initial phase of fibrosis, involve damage to the endothelial cells (EC) caused by infection, immune mediated cytotoxicity, ischemia-reperfusion injury or anti-endothelial antibodies (32).

EC adhesion ligands are up regulated in response to damage. They promote the binding, rolling and migration of inflammatory cells into the interstitial space from where they can infiltrate the tissue. Simultaneously, vascular smooth muscle cells proliferate in response to growth factors resulting in intimal proliferation and matrix deposition in the vessel wall. These processes can eventually lead to complete occlusion of the vascular lumen (33).

The loss of viable ECs also leads to an imbalance in vasoactive factors such as overproduction of the vasoconstrictor endothelin-1 (ET-1) and underproduction of the vasodilators nitric oxide (NO) and prostacyclin. The enhanced apoptosis of EC results in a loss of capillaries, which is easily detectable with nailfold capillaroscopy. These changes are considered to constitute the first steps in the pathogenesis of SSc (32).

In healthy subjects the loss of EC and tissue ischemia induce enhanced production of angiogenic growth factors, especially vascular endothelial growth factor (VEGF), which initiates vasodilatation, proliferation and migration of ECs to form new vessels, i.e. angiogenic sprouting (33).

VEGF levels are increased in both the plasma and the skin of SSc patients, but despite this, angiogenesis is insufficient (34-36). The underlying mechanisms are not fully understood. They seem to involve harmful effects of prolonged exposure to high levels of VEGF (35, 37) and overexpression of a more anti-angiogenetic splice-variant of VEGF (38)

Not only angiogenesis (the building of new vessels from existing vessels) but also vasculogenesis (the building of new vessels from progenitor cells) are impaired in SSc. Patients with digital vascular lesions and high “SSc severity scores” had lower endothelial progenitor cell (EPC) counts in the circulation, suggesting that inadequate recruitment of bone marrow derived circulating EPCs may be related to the reduced vascular repair capacity in SSc. Reduced number of EPCs has also been associated with capillary loss and severe internal organ involvement (predominantly cardiac) as well as with the development of PAH (39-41).

The release of von Willebrand factor (vWF) into the circulation reflects the activity of the vascular disease in SSc (42). vWF is mainly released from the endothelium. In the skin, it is also found in the perivascular and interstitial matrix, which suggests that the local microvessels of the papillary layer of the skin are damaged in SSc. When the high molecular-weight vWF binds to sub endothelial collagen fibres it can form bridges between the sub endothelial matrix and platelet receptors, and thus contribute to platelet adhesion and aggregation, and later to the formation of thrombosis. Through these mechanisms, local vascular damage and the subsequent release of vWF may contribute to local pathogenic processes (43).

Platelets are the major storage and delivery vehicles for both pro- and anti-angiogenic growth factors, including VEGF (33).

ET-1 is predominantly synthesized by ECs and smooth muscle cells. It can regulate the growth of mesenchymal cells and induce collagen and fibronectin production and cell migration. ET-1 is thus one of the main regulators of ECM synthesis and vascular and interstitial remodelling. In SSc, increased plasma ET-1 levels are involved in enhanced vasoconstriction, vascular EC proliferation, smooth muscle hypertrophy and irreversible vascular remodelling (32).

1.4.3 Activation of immune system:

Inflammation: There are several studies examining the different inflammatory cytokines in SSc, often with divergent results. As Th1 lymphocytes mainly produce cytokines that are involved in cellular immune response, such as IFN- γ , TNF- α and IL-2 these are often referred to as “proinflammatory” in SSc. These cytokines of mainly Th1 origin are often elevated in the early stages of SSc. Th2 lymphocytes mainly produce IL-4, IL-13, IL-5, IL-6 and IL-10 and are believed to be more “profibrotic” in SSc. IL-6, IL-4 and IL-13 are able to stimulate the production of collagen, and they can inhibit collagenase synthesis, which favours fibrosis. IL-4 and IL-13 both stimulate B cell differentiation, with consequent production of autoantibodies and isotype switching. Additionally, IL-13 induces TGF- β gene expression (44).

There is evidence of elevated IL-6 levels in the circulation and the skin in patients with SSc. IL-6 has a pro-inflammatory function in the presence of TGF- β / IL-21 via induction of IL-17 differentiation. IL-6 also inhibits the differentiation of T regulatory lymphocytes and stimulates the production of collagen (45).

Additionally it has been demonstrated that more traditional measures of inflammation are important in SSc. Thus an erythrocyte sedimentation rate (ESR) > 25 mm is a predictor of mortality and an elevated CRP, especially prevalent in early dcSSc, is associated with SSc disease activity, severity, and poorer survival (46).

Antibodies: Antinuclear antibodies (ANA) are frequent in SSc, present in >90% of the patients. The most common antibodies in SSc patients are anticentromere antibodies (ACA), antitopoisomerase I antibodies (ATA)/Scleroderma-70 antibodies (Scl-70) and anti RNA polymerase III antibodies (ARA). Presence of one of these antibodies constitutes one of the classification criteria in the new ACR/EULAR classification of SSc (11) The presence of these antibodies has been shown to predict the onset of SSc before a clinical diagnosis is established (47, 48).

About 20-40% of the SSc patients have ACA, but the frequency is depending on ethnicity, with a lower frequency in Afro-Americans and Thai SSc patients. The sensitivity of ACA is in the range of 20-40% and the specificity >90% (49).

ACA have been associated with lcSSc (50, 51), pulmonary arterial hypertension (52, 53) but they seem to have a lower frequency among patients with severe pulmonary fibrosis and renal crisis (54). ACA have also been associated with a more favourable prognosis than other antibodies.

ATA are found in 10-40% of the SSc patients and the specificity can be as high as 99% but the sensitivity is less than 25%. ATA have been associated with a poorer prognosis, pulmonary fibrosis, musculoskeletal, and cardiac involvement (48, 50, 52).

ARA occurs in 10-20% of the SSc-patients and they are associated with dcSSc and a higher risk of scleroderma renal crisis (55, 56).

Sjögren's Syndrome antibodies A and B (SSA/SSB) are found in about 20% of the SSc patient (49).

1.5 CLINICAL FEATURES

There are many different clinical manifestations in systemic sclerosis. The most common are thickness of the skin, Raynaud's phenomenon and gastrointestinal symptoms, such as reflux, bloating and diarrhoea. Arthritis, myositis and/or calcinosis (calcium deposit under the skin) are seen in some patients (57).

About 50% of the patients experience at least one digital ulcer during the disease course. It is not uncommon that ulcers recur and they can lead to digital loss.

The high mortality rate, pooled standardized mortality ratio (SMR) 3.5, observed in SSc is mainly associated with cardiac or pulmonary disease manifestations (58). Pulmonary fibrosis is prevalent in 40-80% of SSc patients depending of diagnosis modality or subgroup. Most cases have a benign course, but about 15% have more widespread pulmonary fibrosis (57).

Several disease manifestations, such as severe pulmonary fibrosis, left sided heart failure, pulmonary embolism and pulmonary arterial hypertension (PAH) can lead to pulmonary hypertension (PH) in SSc. Between 7-15 % have PAH, a severe life-threatening condition, which arises as a consequence of vascular disease in the lungs (57).

1.6 TREATMENT

There are no curative treatments for SSc. Most of the patients have histamine₂-receptor antagonists (H₂ blockers) the gastrointestinal problems and calcium channel-blockers for Raynaud's Phenomenon (59)

Corticosteroids are used in low dosages for shorter periods in some patients, especially in SSc patients with myositis or arthritis. Higher corticosteroid dosages have been associated with the development of SCR and should therefore be avoided (60)

Disease modifying anti rheumatic drugs (DMARD) such as methotrexate are used for in widespread active skin disease and cyclophosphamide and mycophenolate mofetil for alveolitis/pulmonary fibrosis. Endothelin-antagonists, phosphodiesterase-blockers and prostacyclin are used for severe vascular disease such as PAH and also for recurrent digital ulcers (59).

2 CARDIOVASCULAR DISEASE AND ATHEROSCLEROSIS

2.1 ATHEROSCLEROSIS

CVD is the leading cause of mortality in the world and in Sweden about 40% of all deaths are due to CVD (61). Atherosclerosis is considered to be the main cause of CVD. The process of atherosclerosis begins already in childhood with the development of "fatty streaks" in the intima of the vessels. These streaks gradually transform into fibromuscular plaques (62).

The cores of the plaques are filled with lipids and necrotic debris. The plaques are surrounded with a fibromuscular cap, formed by the smooth muscle cells of the media. The plaques grow slowly. Initially they expand outwards with preserved vascular lumen, but with time they can cause narrowing of the lumen, obstruct the blood flow and thus trigger symptoms of ischemia (63). The fibrous cap can also eventually become thin and unstable, resulting in a vulnerable plaque with a high risk of rupture. If rupture occurs, the necrotic debris of the core is exposed to the circulating blood and a thrombus is formed, which can lead to total occlusion of the vessel (64).

Myocardial infarction, ischemic stroke, sudden cardiac death, chronic ischemic heart disease and peripheral arterial disease are the most common clinical manifestations of atherosclerosis.

The atherosclerotic process is a common feature in most individuals, but the progression to vulnerable plaques, or plaques, which obstruct the vessel lumen, are dependent on several factors. During the last 20 years, atherosclerosis has been recognized as a chronic inflammatory disease, where local and systemic inflammation is thought to play an important role (65). The origin of atherosclerosis is believed to take place in the endothelium. Endothelial activation or injury can have varying causes. An activated endothelium is prone to take up oxidized LDL (oxLDL) to be engulfed by subendothelial macrophages that transform into foam cells. Foam cells make up the core of the atheroma, which with time can develop into a culprit lesion (66-68).



Figure 1. Atherosclerotic plaque stable, and vulnerable

2.2 METHODS TO MEASURE ATHEROSCLEROSIS AND ENDOTHELIAL DYSFUNCTION:

One of the most commonly used methods to visualize atherosclerotic plaques/stenoses or occlusions is B-mode ultrasound. One area of particular interest is the carotid arteries because its easily accessible and its bifurcation is a common site of atherosclerosis (69). The intima media thickness (IMT) of the common carotid artery is also used as a surrogate marker for atherosclerosis in coronary vessels (70). An increased IMT is predictive of future CVD (71), although plaque occurrence seems to be even more predictive for MI (72).

Ankle-brachial index (ABI) is used to detect peripheral arterial disease (73) and a low ABI is predictive of both cardiovascular death and CVD (74). Although most studies have evaluated the risk with a low ABI, often defined as a ratio < 0.9 (73, 75), there is also evidence of an elevated risk for CVD with a high ABI > 1.4 (76), probably a marker of stiff arteries. Artery stiffness per se is also associated with CVD (77) and can be measured in the carotids, the aorta or the femoral arteries.

Another method to detect atherosclerosis is with Electron beam computed tomography. This method yields a coronary artery calcium score (78).

Endothelial dysfunction: is believed to be one of the underlying factors involved in the progression towards atherosclerosis (79). Normally the endothelium favours a vasodilatory, anti-thrombotic, anti-inflammatory state with expression of high levels of nitric oxide (NO) and prostacyclin I_2 (PGI_2) and low levels of reactive oxygen species (ROS) and uric acid. A healthy endothelium expresses low levels of vonWillebrand factor (vWf), plasminogen activating factor-1 (PAI-1) and P-selectin and also low levels of intercellular

adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). An activated/dysfunctional endothelium upregulates ICAM-1 and VCAM-1 to facilitate the migration of inflammatory cells across the endothelium, it decreases NO and PGI₂ to contract the vessels and it upregulates vWf and PAI-1 for platelet adherence and activation (80).

Elevated levels of ICAM-1 have in the general population been associated with a higher risk of future myocardial infarction (MI), angina pectoris and coronary death (81). VCAM-1 levels are higher in persons with established atherosclerosis (82) and they are also associated with CVD in SLE patients (83, 84). High levels of vWf are associated with MI (85), stroke (86) and peripheral arterial disease (87).

VEGF, the growth factor for the endothelial cells, is also more abundant in both the atherosclerotic plaque and in the circulation of patients with atherosclerotic lesions (88, 89).

Other methods to measure the endothelial function are various tests of vascular reactivity, where flow-mediated dilatation (FMD) is the most used test because of its non-invasive approach (90, 91). Endothelial function, as assessed with FMD, seems to be impaired in a variety of systemic autoimmune diseases (92).

2.3 MICROCIRCULATION:

The microcirculation refers to arteries with the smallest resistance, arterioles, capillaries, and venules. Whereas the capillary network is essential for the exchange of nutrients and gas between blood and tissue, arterioles are involved in blood flow regulation (93). In the skin, the microvasculature also represents the main organiser of for thermoregulation (94)

Alterations in microcirculatory blood flow or capillary structures have been identified in several disease processes such as sepsis (95) diabetes (96, 97), cardiovascular disease (98) and SSc (99).

These alterations may have different pathogenic background but they can nevertheless all lead to a reduced blood flow, defect tissue oxygenation, decreased tissue nutrition or capillary rarefaction (93).

In the normal population, 40% of the patients with symptoms of heart ischemia, have normal coronary arteries. In 1985, Cannon and Epstein introduced the term 'microvascular angina' for this patient group, in view of what appeared to be an

enhanced sensitivity of the coronary microcirculation to vasoconstrictor stimuli associated with a limited microvascular vasodilator capacity (100). This patient group consist of mostly women. Although the underlying pathogenesis is unknown; some of these patients have signs of endothelial dysfunction, and a scattered pattern on myocardial scintigraphy (98).

The coronary microcirculation can be indirectly studied during coronary angiography, but this is an invasive method. The cutaneous circulation has therefore emerged as an accessible and potentially representative vascular bed to examine the mechanisms of microcirculatory function and dysfunction (93).

The cutaneous vasculature is organized as two horizontal plexuses in the dermis: the upper network (from which capillary loops arise) located in the papillary dermis is connected to a lower dermal–hypodermal network through ascending arterioles and descending venules. Arteriovenous anastomoses, which bypass the capillary circulation, are found in glabrous skin especially in the digits. There is a major neural influence on skin microvascular reactivity, an this is one reason why the skin contains a higher density of nerve fibers than most other tissues. (93).



Figure 2: Digital vessel with afferent and efferent parts. Collaterals in between.

Structural changes in the capillary vasculature are easy to examine in the nailfolds, where the capillaries lie close to the surface arranged in symmetrical loops. This can be done with a simple magnifying device such as a dermatoscope or an ophthalmoscope, together with some immersion oil. More detailed studies of vessel morphology can be performed with a wide-field stereomicroscope or with a digital videocapillaroscope. Outside the periungueal region, where capillaries are perpendicular to the skin surface, videocapillaroscopy can be used to assess capillary density and capillary recruitment. Nailfold capillaroscopy is the most used method to evaluate the microcirculation in SSc (101).

Other techniques such as orthogonal polarization spectral imaging (OPS) and sidestream dark-field imaging (SDF) provide images of the microcirculation with a high level of contrast on organs covered by a thin epithelial layer. OPS and SDF are used when assessing the microcirculation in organs during surgery and especially SDF has emerged as a non-invasive tool to examine the microcirculation in intensive care patients, often in the sublingual mucosa.(102)

Laser Doppler flowmetry (LDF) is commonly used to assess microvascular reactivity, often together with iontophoresis or in combination with post occlusive reactive hyperaemia. Iontophoresis is a non-invasive method of distributing drugs through the dermis based on the transfer of charged molecules using a low-intensity electric current. LDF, together with iontophoresis of acetylcholine and sodium nitroprusside is used to assess microvascular endothelial function (93).

2.4 CARDIAC FIBROSIS:

The cardiac fibroblasts are responsible for maintaining the delicate matrix network of the heart; the epimysium, enveloping the whole cardiac muscle, the perimysium, surrounding bundles of muscle fibres and the endomysium, that surrounds single myocytes. Cardiac fibrosis is associated with an increased deposition of matrix proteins in the myocardium. It could be either a "reactive interstitial fibrosis" that describes an expansion of extracellular matrix in the absence of cardiomyocyte loss, or "reparative fibrosis", that replaces dead or damaged myocardial cells with myofibroblast (i.e. scarring) (103).

The collagen content increases with age and the endo-and perimysial fibers become thicker. This leads to a stiffer ventricular wall and diastolic dysfunction. In the Framingham heart study there was an age-dependent increase in these two measurements (104).

Myocardial fibrosis is normally a slow process that is more prominent in older ages. In an adult healthy heart almost 75% of the myocardial tissue volume is composed by myocytes, but myocytes account for only about 30-40% of the total number of cells in the heart. Endothelial cells, fibroblasts and pericytes are abundant in the myocardium; small numbers of macrophages, mast cells and dendritic cells are also present in the perivascular and interstitial space (103).

2.5 METHODS TO STUDY THE HEART:

ECG: The ECG device detects and amplifies the repetitive waves of electrical changes on the skin that are generated when the heart muscle progress the depolarisation wave from the sinoatrial node, through the atrium, atrioventricular node and then spread over the ventricles, before the next repolarization starts. The ECG technique was introduced around 1870, but it became clinically available 1901 by Willem Einthoven, who was later awarded the Nobel Prize for this invention. He also gave name to the different parts of the ECG: P, Q, R, S, T, U-waves (105).

The 12-lead ECG is commonly available and widely used in most clinical settings. ECGs can visualize the electrical axis of the heart, the cardiac rhythm, disorders of the activation sequences, signs of hypertrophy and signs of myocardial ischemia.

Holter-ECG: is an ambulatory device used to monitor heart rhythm for longer periods of time, most commonly for 24-hours. For practical reason only a few electrodes are used and therefore Holter recordings don't reflect as many parts of the heart as 12-lead ECGs. The advantage of this method is to detect sporadically emerging arrhythmias such as extrasystoles or paroxysmal atrial fibrillations and also to get an association between patient-recorded symptoms and ECG-alterations (106).

Echocardiography: By combining the function of ultrasound and Doppler techniques it is possible to visualize the heart in real time. Echocardiography can give both a multidimensional picture of the structure and morphology of the heart and the function of the chambers and heart valves. It is possible to measure the dimensions of the atria and ventricles, the thickness of the septal and inferior walls, assess the structure and the function of the cardiac valves, and visualize the amount pericardial effusion, -if any (107)

The left ventricular systolic function is commonly measured by determination of the ejection fraction (EF). This can be done by a variety of methods. In this thesis we used a method based on visual estimation and AV plane displacement (108).

Diastole is the time between the closure of the aortic and the closure of the mitral valves. It can be divided into four different phases. The first phase is the

time from the aortic valve closure till the mitral valve opens. It is called the isovolumetric relaxation time (IRVT). Thereafter come the rapid filling, the slow filling and then the atrial contraction. The diastolic function is determined by a combination of measurements from the inflow over the mitral valve; the maximal velocity during the rapid filling (E), the maximal velocity during the atrial contraction (A), E/A, how rapidly flow velocity declines in early diastole (E-wave deceleration time = DT), the IVRT or the E/e' ratio (107)

The cardiac valves can be studied both structurally and functional with colour Doppler, and regurgitations can be graded from the spectral Doppler intensity. Echocardiography is commonly used in SSc as a screening method for pulmonary hypertension. It is possible to calculate an estimated systolic pulmonary arterial pressure by the formula: $4 \times (v_{\text{max-TI}})^2 + P_{\text{HF}}$, where $v_{\text{max-TI}}$ is the maximal velocity of the tricuspidalis insufficiency and P_{HF} is the pressure in the right atrium (107).

Annual screening for PAH is recommended from both American Heart Association and the European Society of Cardiology. The need for screening was highlighted in a study by Humbert et al, who examined 32 patients with PAH, 16 were diagnosed after a regular screening program and 16 patients were diagnosed in routine care. After 8 years 81% of the patients, diagnosed in "routine care" were dead, compared to 31% of the patients in the regular screening program (109).

2.6 BIOMARKERS FOR CARDIAC DAMAGE:

Cardiac Troponins (cTn):

The troponin complex is composed of three protein subunits; troponin T, I, and C. But only troponins T and I have unique cardiac isoforms. Cardiac troponins are bound to actin in cardiac myofibrils, and only a small fraction of these proteins are soluble in the cytoplasm. Ischemia is thought to alter cell membrane integrity, causing rapid depletion of the soluble cytoplasmatic pool, followed by larger and more sustained release of troponin into the circulation. Because of their specificity for cardiac tissue, cardiac troponins have become the main biomarkers for detection of myocardial infarction (110).

Cardiac troponins T (cTnT) and I (cTnI) provide largely identical information,

but while cTnT assays is produced by a single manufacturer, multiple manufacturers make cTnI assays and each manufacturer uses different antibody pairs, so assays are not comparable (110).

There are studies that indicate that cTnI is to a less extent influenced by impaired renal function (111) or damaged skeletal muscle (112).

During the last years new high sensitivity (hs)-cTn assays for the detection of cardiac troponins have become available (113). These have improved the sensitivity but at the cost of lower specificity. Although an elevated cTn mirrors a myocardial injury, the underlying mechanisms are not always an acute myocardial infarction. An elevated cTn could be due to previous myocardial damage or stressful situations such as sepsis, pulmonary embolism, pulmonary hypertension, infiltrative diseases such as sarcoidosis or amyloidosis, cardio toxic drugs etc (114). With the new hs-cTn assays it is also difficult to determine what is a “normal value”. When cTns are examined in older (>70 years), presumably healthy, persons the cut-off for the 99% percentile is higher than in younger individuals, but it still remains as strong predictor for mortality (115, 116).

NT-proBNP:

In order to counterbalance the vasoconstrictive and sodium retaining neurohormones, which are released from the renin angiotensin aldosterone system (RAAS), the heart produces vasodilating antiproliferative hormone like substances called natriuretic peptides (NP). The main natriuretic peptides produced in the heart are atrium natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Both peptides are produced in the atria, but in a chronic stressful situation such as heart failure, the ventricular myocytes are also able to upregulate their production of BNP (117).

Levels of NPs are elevated in disorders with salt and fluid overload and when atrial or ventricular wall tension is increased. They regulate blood pressure and fluid balance, increase endothelial permeability and have an important role in the body's defence against mineralocorticoid-and salt- induced hypertension and plasma volume expansion. They lead to an increased cardiac output and to improved diastolic function. BNP are stored in secretory granulae and it is as a prohormone called proBNP. When released to the blood it is cleaved to BNP and NT-proBNP. NT-proBNP has a longer half- life, making it suitable for detection in the clinic, but both hormones are useful in the detection and follow up of heart

failure (117).

Although mainly used as a marker of heart failure, higher levels of NT-proBNP can also be seen in other conditions such as left ventricular hypertrophy, tachycardia, pulmonary hypertension, myocardial ischemia, hypoxemia, renal dysfunction, advanced age, liver cirrhosis, sepsis and infection(117). Several studies have noticed the usefulness of NT-proBNP in screening and follow up for patients with SSc-PAH and it is now used in many countries as a “clinical routine” test for these patients (118-121)

2.7 OTHER BIOMARKERS

Uric Acid: During the last decades uric acid (UA) has been studied as a possible biomarker for cardiovascular disease (122). Elevated levels of UA have been associated with MI, stroke and heart failure (123, 124). Although many other more established risk factors are associated with higher levels of UA, such as kidney disease, obesity, hypertension, there is some evidence that supports a causal connection between UA and CVD (122).

UA is a product of purine metabolism, formed from the breakdown of adenosine and guanine. It is produced through the action of the enzyme xanthine oxidase; during a process when oxidants are also produced. This could imply that the elevated UA only is a sign of enhanced oxidation, but there are several theories suggesting a harmful effect of UA per se. Local and circulating UA levels are influenced by tissue oxygenation and hemodynamic aspects as well as by dietary, genetic, drug-related, and renal factors. It seems like UA can act both as an antioxidant or oxidant depending on the environment. UA mediates endothelial dysfunction by decreasing NO and induce vascular smooth muscle cells via a pathway involving PDGF. UA can also accumulate inside atherosclerotic lesions (125) and elevated UA is seen in both idiopathic (126) and SSc-PAH (127).

Cystatin C: Cystatin C is a reversible inhibitor of cysteine proteinases that is produced by most cells. It is freely filtered at the glomerulus and is then reabsorbed and catabolized in the proximal renal tubules. It is not affected by gender or muscle mass, and it is sensitive to small impairments of renal function. It is therefore believed to be a more accurate measure of renal function than creatinine, especially when the loss of renal function is moderate (128). In recent years, cystatin C has been reported to be a prognostic marker for CVD in the general population even after adjustment for renal function (129).

2.8 TRADITIONAL RISK FACTORS:

The classical traditional risk factors for cardiovascular disease were described in the large epidemiological study in Framingham 1971. The original Framingham risk factors are age, male gender, smoking, family history, diabetes, hypertension and hyperlipidaemia (130)

There are only a few studies addressing the lipid profile in systemic sclerosis. Most of these studies did not find any differences in lipid profile between patients and controls but there are a few reports of higher levels of triglycerides, lower levels of high density lipoprotein (HDL) (131), higher sensibility to oxidation of low-density lipoprotein (LDL)(132) and higher levels of lipoprotein a (Lp(a)) (133).

3 CARDIOVASCULAR DISEASE AND ATHEROSCLEROSIS IN SYSTEMIC SCLEROSIS

3.1 THE HEART IN SYSTEMIC SCLEROSIS:

Clinical manifest/symptomatic cardiac disease has a prevalence between 15-35% in SSc, but subclinical cardiac disease is found in the majority of patients (134). When clinically evident, cardiac manifestations are associated with a poorer prognosis and mortality (135). All structures of the heart can be involved with manifestations such as pericardial effusion, arrhythmias, conduction system defects, valvular impairment, myocardial ischemia, myocardial hypertrophy and heart failure (136).

In autopsy studies d'Ángelo (137) and Bulkley (138) found patchy myocardial fibrosis with a characteristic distribution of the lesions in the myocardium that did not correspond to the regional blood supply of a single coronary artery. The coronary arteries in these autopsy cases were also strikingly unaffected. In another autopsy study (139), eight SSc patient, all with previous known heart disease (six of them died of sudden death) had distinct morphological abnormalities in the conduction system, especially in the sinus node. Narrowing and platelet-fibrin clots in the small coronary arteries supplying these regions were also detected. This led to the theory of a "myocardial Raynaud's phenomenon", according to which damage to the heart is caused by repeated ischemia-reperfusion. This theory has been tested in several cold-provocative studies (140, 141).

Myocardial biopsies on the other hand have been undertaken in patients with cardiac failure and they often show an inflammatory pattern, i.e. evidence of myocarditis. Most are case reports or case series (142, 143) but in one study comparison between idiopathic PAH and SSc PAH showed a marked inflammation in myocardium of SSc-patients (144).

Clinical myocarditis is commonly associated with skeletal muscle diseases or scleroderma-myositis overlap (145, 146). Pericarditis is prevalent in 30-75% of patients, but it is often asymptomatic (147).

Cardiac MRI has emerged as a sensitive technique to identify cardiac disease in SSc and it has identified a high prevalence of disease, which correlates with previous autopsy studies. Gadolinium-delayed contrast enhancement is used to evaluate myocardial fibrosis while T2-weighted imaging identifies inflammatory

lesions (148-150).

Left ventricular systolic or diastolic dysfunction can occur many years before becoming clinically evident (147, 151). Systolic dysfunction seems to be less common than diastolic dysfunction (152-156) and it mainly occurs together with and likely because of concomitant coronary artery disease or systemic hypertension (152, 157). One study used a newer strain method to detect LV systolic impairment and found LV systolic dysfunction in even in patients with a normal LVEF (158). When a low LVEF is present, it seems to be associated with clinical characteristics such as myositis, digital ulcerations, male gender, older age and it is more common in patients who are not treated with calcium-channel blockers (159).

LV diastolic dysfunction has been subject of numerous studies in SSc because of the assumption that it mirrors myocardial fibrosis. The prevalence of diastolic dysfunction in SSc, measured with conventional Doppler echocardiography, is 20-40% (153, 156, 160, 161), and with tissue Doppler as high as 60% (162, 163), but there are also studies, including ours, where no difference in diastolic dysfunction between patients and controls were detected, especially after adjusting for other factors (164, 165). Although diastolic dysfunction can be detected with several measurements on echocardiography, Lee et al found that the E/e' ratio was more sensitive than E/A ratio in SSc patients (166).

Right ventricular systolic and/or diastolic dysfunctions are also impaired in SSc (167-169), and seem to exist despite no underlying PH or LV dysfunction (156, 164, 170). Right ventricular dysfunction seems furthermore to be more pronounced in patients with SSc-PAH than idiopathic PAH (171, 172).

Echocardiography is used as a screening method for PH in SSc, and an ePAP > 36 mmHg is a predictor of mortality in SSc patients (173). Although right heart catheterisation remains as the golden standard for the detection of PH, echocardiography, especially in combination with other test such as pulmonary function test and biomarkers seems to be reliable for screening (174).

There are not many studies addressing valvular defects in SSc. In autopsy studies, shortening of the chordae tendineae of the mitral valve has been noted, as well as mitral and tricuspid valve vegetations (138). Even if there are several echocardiographic studies in SSc, there are only a few reports on valve regurgitations or stenosis. In one small study including 11 patients with progressive systemic sclerosis, mitral valve prolapse was recorded in 2 patients (175). Kazzam et al examined 30 patients and 30 controls and found mitral regurgitation in 67% of the SSc patients versus 15% of the controls (176). In a study comparing SSc-, myositis-, SLE- patients and controls, patients with SSc had the highest frequency of mitral and/or aortic regurgitation, 10% versus 1.7% in SLE and 0% among myositis patients and the controls. 11,3% of the SSc patients and 18,7% of the SLE patients had tricuspidalis regurgitations; this finding was associated with a higher right systolic pulmonary pressure (177). In a large study consisting of 570 patients, 7.2% had mitral regurgitation, and 2.4% aortic regurgitation (161)

Conduction defects and arrhythmias:

An abnormal ECG is present in 25-75% of patients with SSc and is considered an independent predictor of mortality (178, 179). In a EUSTAR database study, 26% of the deaths were due to cardiac disease and about half of these were due to malignant arrhythmias (135)(Tyndall). In another study cardiac arrhythmias were one of seven variables predictive for mortality, with a hazard ratio (HR) of 2.8 (180).

In a study by Ferri et al, ventricular arrhythmias were found in 90% of SSc patients with Holter ECG, but they did not correlate with clinical variants or with other clinical symptoms or signs of the disease. Abnormal ventricular arrhythmias were more likely in patients with echocardiographic abnormalities (181). A large observatory study reported that severe cardiac arrhythmias with a poor prognosis were significantly more frequent in patients with concomitant skeletal and cardiac muscle involvement (146). Some studies have also demonstrated impairment of heart rate variability and turbulence as a measure of autonomic nervous system dysfunction (182).

Conduction alterations are more prevalent in SSc than controls (183). The most common alteration seems to be left bundle branch block (LBBB), but AV-conduction defects and right bundle branch block (RBBB) are also present. RBBB are predictive for mortality in early SSc (184)

QTc prolongation, which can lead to life-threatening tachy-arrhythmias, has also been reported in SSc patients (178).

Follansbee et al found septal infarction pattern in 10 of the SSc patients but none of the controls. A more detailed examination was done in 6 of these patients and all had septal thallium perfusion abnormalities, but normal coronary angiography (183).

3.2 MACROVASCULAR DISEASE AND ATHEROSCLEROSIS IN SSc

During the last years, there is emerging evidence of a high prevalence in SSc of disease manifestations commonly associated with atherosclerosis such as myocardial infarction (MI), peripheral vascular disease (PVD) and stroke.

During 2012-2013 there were 3 population-based studies addressing this matter from three different continents. In a study from Taiwan, the hazard ratio (HR) for myocardial infarction (MI) in SSc was 2.45 (185) and in a study from Boston, USA, the HR was 1.8 for MI, 2.6 for stroke and 4.35 for PVD (186). In a study from Australia, the odds ratio (OR) for coronary heart disease in SSc was between 1.9 and 3.2 depending on control group and adjustment for other factors (187).

Although the underlying factors still are under evaluation there is some evidence of an enhanced atherosclerosis in SSc patient as compared with controls, but the studies are conflicting and differ in selection of both patients and controls (table below). Two meta-analyses have concluded in favour of enhanced atherosclerosis (188, 189).

Table: Atherosclerosis in systemic sclerosis:

	Characteristics patients	Characteristics controls	IMT (mm) and/or Plaque (%)	
Lekakis et al 1998	N: 12, age: 49, female 100% dcSSc 100% No ACA	N: 12, age: 49 female 100% Healthy, no ECG abnormalities	0.83 ± 0.3 patients 0.46 ± 0.2 controls	↑ P=0.002
Stafford et al 1998	N: 20, age: 64, female 90% lcSSc 90% dcSSc10%	N: 20, age: 66 Other reumatic diseases (excluding SLE and vasculitis) matched for age, sex, smoking, diabetes, and hypertension	CCA diameter cm/m ² 43.3 patients 44.8 controls	↔ p=0.7
Cheng et al. 2003	N: 53, age 55, females 81%	N: 20, age 43 females 80% Patients undergoing surgery, no cardiac disease	0.68 ± 0.27 (lcSSc) 0.62±0.20 (dcSSc) 0.63 ± 0.20 controls	↔ P = 0.090
Szucs et al. 2007	N: 29, age 52 Excl: previous CVD, diabetes, smokers, BMI>30, vasculitis, infection, renal failure.	N: 29, age 49 Health staff and patients from the hospital, matched for age, sex and traditional risk factors	0.67±0.26 patients 0.57± 0.09 controls	↔ P = 0.067
Kaloudi et al. 2007	N: 66, age 62 55 lssc, 11 dssc Excl: diabetes, smokers, renal failure.	N: 20, age 58 Selection not specified	0.90 ± 0.04 (lcSSc) 0.87 ± 0.04 (dcSSc) 0.69 ± 0.01 controls	↑ P < 0.01
Bartoli et al 2007	N: 53, age 60 females 89% lcSSc 85% dcSSc	N: 53 age: 56, females 93% Excl: previous CVD	0.85 ± 0.03 patients 0.68 ±0.01 controls	↑ P<0.03
Bartoli et al. 2007	N: 35, age: 61 Excl: diabetes, kidney disease, tx: steroids or Cyc.	N: 20 age not specified Health staff, comparable age, sex, traditional risk factors.	0.93 ± 0.29 patients 0.77 ±0.13 controls	↑ P<0.003
Zakopoulos et al. 2003	N: 40, age not specified	N: 45 age not specified	0.69±0.25 patients 0.59±0.1 controls	↔ NS
Roustit et al. 2008	N: 42, age 51 Excl: smoking, diabetes, severe disease (Cardiac or pulmonary failure, IHD)	N: 33, age 52 Asked by advertice in paper	0.53±0.23 patients 0.51±0.11 controls	↔ p=NS
Hetteema 2008	N: 49, age 55, females 84% lcSSc 82%, dcSSc 8%	N: 32, age 51, females 91% Selection not specified.	0.69 0.68	↔ p = 0.067
Ho et al. 2000	N: 54, age: 57, female 93% lcSSc, dcSSc	N: 43, age: 53, female 81% Population-based with comparable age, gender. Excl: Raynaud's phenomenon, other Rheumatic diseases	64% patients 35% controls	↑ p= 0,007
Sherer et al. 2007	N: 44, age: 62, gender ? lcSSc 86%, dcSSc 14% ACA 50%	N: 32, age: 60, gender ? Age-matched	IMT > 0.9 mm: 43% patients 28% controls	↑ P=0,026
Tsifetaki et al 2010	N: 60, age 56, females: 92% lcSSc Excl: history of CVD, hypertension, smokers, diabetes, hypothyroidism, liver or kidney diseases, Cushing's syndrome, BMI > 30, receiving medications affecting lipid metabolism	N: 51, age 51, females 88%	0.77 ± 0.2 patients 0.59 ± 0.14 controls	↑ p<0,0001
Vettori et al. 2010	N: 50, age 52, females 92% lcSSc 70%, dcSSc 30 % ACA 52%	N: 41, age: 52, females 90% Fibromyalgia or osteoarthritis patients matched for age and sex.	0.61 ± 0.24 patients 0.65 ± 0.17 controls IMT >9 and/or plaque 28% patients 9.8% controls	↔ P>0.05 ↑ P=0.004
Schiopu et al 2013	N: 46, age: 49, females 100% lcSSc 50% dcSSc 50%	N: 46, age: 49, females 100% Matched for age and ethnicity, randomly selected from a previous study consisting of 167 controls, reported healthy.	0.59±0.13 patients 0.56±0.13 controls plaque: 46% patients 20% controls	↔ P=0.07 ↑ P=0.01

N: numbers, SSc: Systemic sclerosis, IMT: intima media thickness, dcSSc: diffuse cutaneous SSc, lcSSc: limited cutaneous SSc, ACA: anticentromere antibodies, SLE: systemic lupus erythematosus, CCA: common carotid arteries, CVD: cardiovascular disease, IHD: Ischemic heart disease, BMI: body mass index

References:(190-204)

3.3 MICROVASCULAR DISEASE IN SYSTEMIC SCLEROSIS:

1975 Campbell and LeRoy presented their “vascular hypothesis”, which suggested that microvascular disease was a fundamental part of the pathogenesis of scleroderma (205). This hypothesis has led to a change in the definition of SSc criteria (11) and also to development of more vascular animal models addressing the pathways involved in the progression to both vasculopathy and fibrosis (37, 206, 207).

Raynaud’s phenomenon (RP) is one of the most common disease manifestations in systemic SSc, affecting >90% of all patients. In most cases it also precedes the other disease manifestations by several years. In addition to an injury of the endothelium, decreased release of vasodilatory neuropeptides from sensory nerves and up regulation of vascular smooth muscle receptors that enhance vasoconstrictive responses to stress or cold stimuli are implicated in the dysfunction leading to RP (32).

Biopsies from the digits of patients with SSc-RP shows unique changes in vascular structure, characterized by intimal thickening, narrowing of the vascular lumen and in some cases also microthrombosis (208).

The structural changes in the capillaries are easily detected in the nail folds of SSc patients and a typical scleroderma pattern has been described, which includes capillary loss, dilated capillaries, microhemorrhages (209). This pattern is present in >80% of the SSc patients, but its not unique for SSc. It can occasionally be found in other connective tissue diseases, such as mixed connective tissue disease (MCTD) or myositis (210). A staging system has been developed by Cutolo et al dividing the morphological changes into an early, active and late pattern (99).

The structural changes in the capillaries can be visualised in other sites of the body as telangiectasias in the skin or gastric antral vascular ectasias (GAVE) in the stomach (32).

Although the morphological changes in the nailfold capillaries are most studied, there are also evidence of impaired capillary blood flow (211) and a reduced response to different stimuli with iontophoresis (212).

The changes in nailfold capillary pattern, especially loss of capillaries, has been associated with disease severity (213), an elevated ePAP or PAH (214-216), digital ulcers (217), severity of digital ischemia and ACA (218).

About 40-50% of the SSc patients develop digital ulcers, usually in the first 5 years from disease onset (61). Digital ulcers localized on the finger pads can leave a pitting scar, which can be seen in 15-30% of the patients. The ulcers take 3-15 months to heal (219) and are associated with a reduced quality of life, work disability (220). Infections, osteitis, gangrene and amputations are some of the complications associated with DU in SSc (221).

Digital ulcers can also be seen on the dorsum of the hand, often localized on the knuckles. These ulcers seem to be more associated with mechanical stress because of the tightness of the skin and contractures in this region. Despite this, these ulcers reduce QoL just as much (222).

Digital ulcers have been associated with capillary microscopy findings and reduced capillary density is therefore used in two different score system for prediction of future digital ulcers (223, 224). Factors associated with digital ulcers are many and in several studies discrepant i.e. male gender, dcSSc, ATA (52, 219, 225) or lcSSc, and ACA(226, 227) are factors which have been positively associated. Because of the discrepant in definition of digital ulcers (228), an international group has suggested a method of categorizing ulcers for pharmacological studies(229).

Besides RP and digital ulcers there are two other severe disease manifestations where the vasculopathy seems to be of major importance: the scleroderma renal crisis (SRC) and pulmonary arterial hypertension (PAH) (32).

SCR was the major cause of death in SSc about 40 years ago, but since the introduction of ACE inhibitors, this disease manifestation is rare (230). SRC is typically characterized by a sudden and marked increase in systemic blood pressure (although normotensive SRC has been described) and acute renal failure (230). The histopathology shows a thrombotic microangiopathic process similar to idiopathic malignant hypertension with small vessel changes that predominate over glomerular alterations (231). Small vessel thrombi, intimal fibrosis and an onion-like appearance around the vessels, which obstruct the lumen, are typical for SRC. Even if early treatment with ACE-inhibitors have reduced mortality, SRC is associated with significant morbidity and mortality (232, 233)

PAH is together with pulmonary fibrosis the dominating cause of scleroderma related deaths (ref). Despite new PAH treatments during the last 10 years, SSc associated PAH still has a more pessimistic prognosis than idiopathic PAH.

4 AIMS

We wanted to examine if SSc patients had more macrovascular disease, atherosclerosis and cardiac disease than controls, and also if macro- and microvascular disease were associated in SSc patient.

We specifically aimed to answer the following questions:

1. Are macrovascular events more common in SSc patients than in controls, and if so, what events are more prevalent?
2. Do SSc patients have more atherosclerosis than controls?
3. How do traditional risk factors, inflammatory and endothelial biomarkers relate to the occurrence of macrovascular disease and atherosclerosis in SSc?
4. Do cardiac rhythm disorders differ between SSc patients and controls?
5. Do echocardiographic abnormalities differ between SSc patients and controls? Are echocardiographic abnormalities associated with SSc subsets?
6. Are the cardiac biomarkers (cTnI, NT-proBNP) elevated in SSc patients, and are they associated with echocardiographic abnormalities?
7. Are there any associations between macrovascular and microvascular disease in SSc?

5 METHODS

5.1 STUDY POPULATIONS

Studies I-III are cross-sectional case-control studies, including 74% of the known prevalent SSc-cases in Stockholm County. Study IV is a cross sectional study including 163 consecutive SSc patients.

Studie I-III.

All participants were >18 years old and recruited from the adult population in Stockholm County (N=1 534 272) between August 2006 and December 2009.

Patients:

Prevalent cases with SSc were identified at the three Rheumatology clinics associated with general/university hospitals and from all rheumatologists with private practice. All dermatologists, gastroenterologists, specialists in respiratory medicine, hand surgeons, cardiologists, vascular surgeons and general practitioners were contacted twice and asked to inform us about their patients with SSc or suspected SSc. A letter describing features of SSc was attached.

Through this approach, we identified 149 cases fulfilling the 1980 ARA criteria for SSc (7), which corresponds to a prevalence of 97 adult SSc cases/ million. Of those 118 patients accepted to participate in the study and were included. Seven patients did not complete the cardiovascular examination, and one patient was only investigated with carotid ultrasound but not with the heart examination, therefore study I consists of 111 patients and study II and III consist of 110 patients. After permission from the patients not included in the study (26%), their medical records were reviewed, and they did not differ from the included patients (74%) regarding age, disease duration or severe organ manifestations.

We also performed a search for the diagnosis M34.0-M34.9 in the Swedish inpatient/outpatient registry for persons living in Stockholm between august 2006 and December 2009. This search identified 235 patients with one of these diagnose codes, but earlier studies have demonstrated a high level of misclassification in this diagnosis group, with about 50 % of the patients not fulfilling the 1980 ARA criteria. (14, 234).

Controls:

Control subjects were recruited from the same population through use of the national registration number, which includes date of birth and is coded for sex. The sex-matched person with the birth date closest to the patient was contacted and asked to participate. A diagnosis of SSc was the only exclusion criteria. When the “first choice” declined, the second closest person was asked, until a control subject gave his/her consent. In 65 % of the cases, the “first choice control” accepted to participate. 110 controls were included, but five did not complete the cardiac/carotid examination, thus 105 controls participated in study I-III.

Studie IV

The 118 patients included between 2006-2009 and 45 patients included between January 2010-September 2013 participated in this study. We used the new classification criteria for this cohort (11) thus including 11 patients who only fulfilled the new classification criteria. The rest of the patients fulfilled both the 1980 ARA and the 2013 ACR/EULAR classification criteria. Some of the patients, who did not want to participate in studies I-III, gave their consent to participate in study IV.

The local Ethics Committee of the Karolinska University Hospital approved the studies and all participants gave written informed consent.

5.2 DEFINITION OF DISEASE ACTIVITY, SEVERITY, ORGAN INVOLVEMENT AND CARDIOVASCULAR EVENTS:

Table 4: European Scleroderma Study Group activity score (235).

Criteria	Point
mRss >14	1
Scleredema	0.5
Deterioration skin*	2
Digital necrosis (active digital ulcers ranging from digital tip infarcts to digital gangrene)	0.5
Deterioration vascular*	0.5
Arthritis	0.5
DLCO<80% of predicted value	0.5
Deteriotation heart/lung*	2
ESR >30	1.5
Hypocomplementemia (Low C3 or C4)	1

mRSSc: modified rodnan skin score, deterioration*: patient report worsening the last month of these symptoms.

DLCO: Diffusion capacity of the lung for carbon monoxide. ESR: erythrocyte sedimentation rate.

A score >2.5 is defined as active disease.

Table 5: Medsger Disease Severity scale: (236)

Organ system	0	1 (Mild)	2 (Moderate)	3 (Severe)	4 (Endstage)
General		Wt loss 5.0–9.9 kg PCV 33.0–36.9%	Wt loss 10.0–14.9 PCV 29.0–32.9	Wt loss 15.0–19.9 PCV 25.0–28.9	Wt loss 20.0+, PCV < 25.0
Peripheral vascular		Raynaud phenomenon requiring vasodilators	Digital pitting scars	Digital tip ulcerations	Digital gangrene
Skin		TSS = 1–14	TSS = 15–29	TSS = 30–39	TSS = 40+
Joint/tendon		<i>FTP = 1.0–1.9</i>	<i>FTP = 2.0–3.9</i>	<i>FTP = 4.0–4.9</i>	<i>FTP = 5.0+</i>
Muscle		<i>Proximal weakness, mild</i>	<i>Proximal weakness, moderate</i>	<i>Proximal weakness, severe</i>	<i>Proximal weakness, severe; ambulation aids required</i>
Gastro--intestinal tract		Distal esophageal hypoperistalsis; small bowel series abnormal	Distal esophageal aperistalsis; antibiotics required for	Malabsorption syndrome; episodes of pseudoobstruction	Hyperalimentation required
Lung		DLCO 70–80%, FVC 70–80%, rales, fibrosis on ograph	DLCO 50–69%, FVC 50–69%, mild PH	DLCO < 50%, FVC < 50%, mod-sev PH	Oxygen required
Heart		EKG conduction defect LVEF 45–49%	Arrhythmia RVE plus LVE, LVEF 40–44%	LVEF < 40%	CHF, arrhythmia requiring Rx
Kidney		Serum creatinine 1.3–1.6 mg/dL Urine protein 2+	Serum creatinine 1.7–2.9 Urine protein 3–4+	Serum creatinine 3.0+	Dialysis required

Wt: weight, CHF, congestive heart failure; DLCO, diffusing capacity for carbon monoxide, % predicted; ECG, electrocardiogram; FVC, forced vital capacity, % predicted; FTP, fingertip-to-palm distance in flexion; LVE, left ventricular enlargement; LVEF, left ventricular ejection fraction; PCV, packed cell volume (hematocrit); RVE, right ventricular enlargement; TSS, total skin thickness score.

We omitted the joint/tendon and muscular scores due to lack of data regarding these items.

Organ involvements were defined as:

Skin score: measured by modified Rodnan skin score (237). This is a method of manually measure the skin by palpation the skin to assess the structure. A scale from 0-52, where 0 is normal structure and 3 is adhered to the underlying tissue. Skin thickness is measured at 17 sites.

Suspected pulmonary hypertension (sPH): tricuspidalis V-max >2.9m/s on Doppler ultrasound.

Pulmonary fibrosis: signs of fibrosis on X-ray or high-resolution computed tomography (HRCT). HRCT was performed in 90% of the patients.

Myositis: muscular weakness and elevated creatine kinase (CK) and signs of inflammation on magnetic resonance imaging (MRI), electromyography (EMG) or muscle biopsy.

Arthritis: swollen joints on examination or arthritis documented in the journals.

Kidney involvement: a history of scleroderma renal crisis or >1 on the Medsger scale for kidney damage

Digital ulcers: a history of digital ulcers documented in the journal or present at inclusion

Calcinosis: calcium deposits in the skin, observed at inclusion or vizualised on X-ray.

Raynauds Phenomenon: Self reported, with at least a 2-phase colour change in fingers consisting of pallor, cyanosis and/or hyperemia in response to cold exposure or emotion.

Scleroderma Renal Crisis: A sudden and marked increase in systemic blood pressure and acute renal failure.

Ischemic arterial events (IAE) were defined as:

Ischemic heart disease (IHD): myocardial infarction (confirmed by electrocardiography and a reversible rise in plasma creatine kinase, muscle and brain fraction (CK-MB) or troponin T) or angina pectoris (confirmed by an exercise stress test).

Ischemic cerebrovascular disease (ICVD): cerebral infarction (confirmed by computed tomography) or transitory ischemic attacks (TIA, defined as transient focal symptoms from the brain or retina with a maximum duration of 24 hours).

Ischemic peripheral vascular disease (IPVD): intermittent claudication + ankle-brachial index (ABI) <0.9 or peripheral arterial thrombosis/embolus (confirmed by angiogram or Doppler flow studies).

Any ischemic arterial event includes 1 to 3 above.

5.3 ASSESSMENT OF ATHEROSCLEROSIS, HEART RHYTHM, HEART FUNCTION AND MICROVASCULAR STRUCTURE:

Study I:

Carotid ultrasound:

The left and right common carotid arteries and bifurcation areas were scanned for presence of plaque and images for IMT measurements were received using a duplex scanner (Siemens Acuson Sequoia, Mountain View, CA, USA) with a 7.0 MHz linear array transducer. Scans were digitalized for offline analysis. The subject's head was tilted to get the picture of the common carotid artery (CCA) just proximal to the bulb placed horizontally across the screen. Pictures were frozen synchronously with the R wave on the electrocardiogram.

The IMT was defined as the distance between the leading edges of the luminal echo and the media/adventitia echo. The IMT was calculated as the intima-media area divided by the measured length (10mm) on one scan.

Plaques were defined as a local increase in wall thickness of >1 mm and 100% increase in wall thickness compared to the adjacent wall.

Ankle-brachial index:

The study persons were placed in a supine position. The highest systolic pressure obtained in arteria tibialis posterior or (if tibialis posterior was inaccessible) in arteria dorsalis pedis for each foot were divided by the highest brachial systolic pressure to obtain an ankle-brachial pressure ratio (ABI).

Study II:

Standard and Holter ECGs:

All patients and controls underwent a standard 12-lead ECG. The first participants (49 patients, 42 controls) were investigated at the Department of Clinical Physiology, Karolinska University Hospital; they underwent a 22–24-h ECG Holter recording (Aspect, Danica Biomedical AB, Borlänge, Sweden). The remaining participants (61 patients, 63 controls) were investigated at Aleris FysiologLab, Sophiahemmet; they were not subject to Holter tracings. A single experienced reader interpreted the recordings without knowledge of patient/control status or other test results.

Atrioventricular (AV) and intraventricular (IV) conduction defects were defined according to the Minnesota Code Manual (238) Left axis deviation in the horizontal plane was defined as an electrical axis between 0° – 90° . Left ventricular (LV) hypertrophy was defined as an R wave in V5 ≥ 26 mm or R wave in V5 plus an S wave in V1 ≥ 35 mm, ventricular activation time in V5 ≥ 0.05 s, and ST-T depression in V4–V6. ST segment depression was defined as > 1 mm horizontal or down-sloping depression.

Abnormalities on Holter recordings were defined as:

Ventricular tachycardia, complex ventricular ectopic beats or solitary ventricular ectopic beats > 100 for subjects below 50 years of age or > 200 for subjects over 50 years of age. Supraventricular tachycardia (SVT) was considered abnormal for subjects over 50 years of age if > 10 beats, or if more than two episodes/24 h. In subjects aged < 50 years, the presence of any SVT was considered abnormal. Solitary supraventricular beats were considered abnormal if > 1000 in subjects over 60 years of age, > 100 in the 40–60-year age group and > 10 in subjects under 40 years of age.

Study II and III

Echocardiography:

We used an Acuson Sequoia (Mountain View, California, USA) with a 2.5 or 3.5 MHz transducer or a GE Vingmed system 7 (Horten, Norway).

Two-dimensional measures were taken as recommended by the American Society of Echocardiography (239). Measures of wall thickness and left ventricular (LV) plane displacement (240). Briefly atrioventricular plane displacement was measured from the apical 4 and 2-chamber views, the M-mode cursor was placed at the septal, lateral, anterior and posterior border of the LV AV plane (mitral ring) and at the AV plane of the right ventricular free wall. The mean AV plane displacement was the average calculated from three heart cycles from the four different M-mode positions.

The valves were studied carefully for valve thickening and other malformations. Doppler and colour Doppler was used to assess valvular stenosis and/ or leakage. Regurgitations were graded from the spectral Doppler intensity, the width of the colourjet at the base and the appearance of the colour Doppler jet. Regurgitation was graded from 1–4 where 1 is mild and 4 severe and regurgitation was considered present if it was grade 1 or more. Valvular abnormalities were

classified as either abnormal localized echodensity adjacent to valve leaflets or valve thickening.

Pulmonary artery pressure was estimated by continuous wave Doppler measurement of the peak systolic velocity of the tricuspid regurgitation. We used the following criteria for PH:

1. Tricuspid regurgitation velocity > 2.9 m/s, corresponding to an estimated pulmonary artery pressure (ePAP) > 34 mmHg at rest with or without additional echocardiographic parameters suggesting PH like a dilated right ventricle (RV) and impaired RV function.
2. Tricuspid regurgitation velocity < 2.9 m/s but additional echocardiographic parameters suggesting pulmonary artery hypertension. In the absence of tricuspid regurgitation pulmonary artery pressure was considered normal. RV function was considered abnormal if the free right chamber wall AV movement was < 17 mm. Tissue Doppler measurement were made at the septal basal segment of the left ventricle and the systolic velocity and the diastolic E and A wave velocities were measured.

Study IV

Widefield nailfold capillaroscopy:

A stereo-zoom microscope instrument (Olympus) set at 20 times magnification and equipped with a transparent ruler in one of the eyepieces was used. All nailfolds except the thumbs were examined for presence of microhemorrhages, enlarged capillary loops and avascular regions. The capillary density (number of capillary loops/3mm) was defined as the mean capillary density in the distal row in the center of the fourth finger on both hands, because the fourth fingers are reported to be the best evaluable fingers (241). Enlarged capillary loops were defined as >4 x ordinary capillaries and avascular areas were defined as a lack of >2 capillary loops (242).

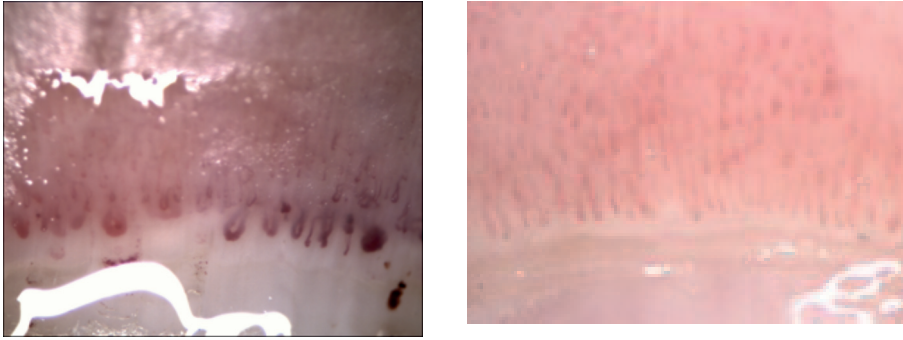


Figure 4: Nailfold capillary findings in a patient with SSc (A) and a healthy individual (B). The photos are provided by Marie Wildt, Department of Rheumatology, Skåne University hospital, Lund.

Laboratory parameters:

Paper I: High-sensitivity C-reactive protein (hsCRP), α -1 antitrypsin, orosomucoid and fibrinogen were measured using BN ProSpec System (Dade Behring, Deerfield, IL, USA). Intercellular adhesion molecule 1(ICAM-1), vascular cell adhesion molecule 1(VCAM-1), interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) were analysed by sandwich ELISA kits (DY720, DY206, DY137 and DY293B, R & D Systems, Minneapolis, MN, USA). Cut-off values were 15 pg/mL for ICAM-1, VCAM-1 and VEGF and 6 pg/mL for IL-6. von Willebrand factor (vWF) was measured by sandwich ELISA (Dako, Glostrup, Denmark) [16].

Paper I and III: Cystatin C (reagent: 1014, Gentian, Moss, Norway) was analysed on an Architect Ci8200TM analyser (Abbott, Abbot Park, IL, USA). The total analytical imprecision of the cystatin C method was 1.1% at 1.25 mg/L and 1.4% at 5.45 mg/L. The equation used for calculating glomerular filtration rate (GFR) in mL/min/1.73 m² from the cystatin C results in mg/mL was $y = 79.901x - 1.4389$ [15].

Paper II: Apolipoprotein (Apo)A1 and ApoB were measured on an Architect Ci8200 analyser (Abbott Laboratories, Abbott Park, IL, USA).

Paper III: High sensitivity cardiac Troponin I (reagent 3P23) and uric acid (reagent 3P39-21) were measured with an Architect Ci16200® analyser (Abbott

Laboratories, Abbot Park, IL, USA). The limit of detection of the Troponin I assay was 2 ng/L and the total coefficient of variation (CV) was 5.5% at 22 ng/L and 4.4% at 200 ng/L. The uric acid method had a total CV of 2.1% at 280 μ mol/L and 0.7% at 580 μ mol/L.

NT-proBNP was measured by a Roche Cobas 8000, using the e602 module (Roche Diagnostics, Mannheim, Germany) according to the specifications of the manufacturer. The instrument had a total CV of 0.9% at 107 ng/L and 1.3% at 2060 ng/L.

Autoantibodies:

Paper I-IV: Antinuclear antibodies (ANA) were analysed by immunofluorescence (IFL) on sections of rat liver and HEp-2 cells (Immunoconcepts, Sacramento, CA, USA).

Paper I, II and IV: Anticentromere antibodies (ACA), anti-topoisomerase 3 (ATA), and anti-Sjögren's syndrome antigen A (anti-SSA) Ro-52 were analysed by the multiplex immunoassay BioPlex 2200 ANA screen system (Bio-Rad, Hercules, CA, USA).

Paper III: A line immunoassay (Euroline, Euroimmun Lübeck, Germany) was used to detect IgG-antibodies to Scl-70, CENP A, CENPB, RP11, RP155,

Statistics (I-IV)

Characteristics of the study populations are described using descriptive statistics: for continuous variables, mean \pm standard deviation (SD) are used when normally distributed, otherwise median and interquartile range are presented. Categorical variables are presented as percentages. Skewed continuous variables were log transformed to obtain a normal distribution, if possible. Groups are compared using ANOVA or Mann-Whitney U test for continuous variables and χ^2 or Fischers exact test for categorical variables.

Odds ratio (OR) and 95% confidence intervals (CI) are calculated from 2×2 contingency tables or from nominal logistic regression models. For continuous variables, we used standard least squares linear regression to calculate standardized regression coefficients (b) and P values. In paper I variables are sorted into functional groups (traditional risk factors etc.). After age adjustment,

the variables within each group, which were representative and most significantly, as determined by lowest P value, associated with the outcome were entered into a multivariable-adjusted model. Due to limited number of observations, we restricted the number of variables. Calculations were performed using JMP software SAS Institute, Cary, NC, USA). A P value < 0.05 was considered statistically significant.

6 RESULTS:

Paper 1:

We examined the presence of subclinical atherosclerosis in 111 SSc patients and 105 population based controls, individually matched to the patients for age and gender. The IMT, frequency of plaque and the ABI were compared. We also examined the presence of ischemic arterial events (IAE) i.e. ischemic heart disease (IHD), ischemic peripheral vascular disease (IPVD) and ischemic cerebrovascular disease (ICVD).

Patients with SSc had a history of more IAE than controls, especially IHD and IPVD. Despite this we found no difference between patients and controls regarding any of the surrogate markers for atherosclerosis on a group level. SSc patient had generally higher levels of biomarkers for inflammation and endothelial activation and also higher triglycerides than controls. The patients had a lower BMI and lower diastolic blood pressure than controls.

We found that the ACA+ SSc subgroup had a history of more IAE, IPVD and they also had more plaques than controls. Subgroup analyses revealed that the ACA+ patients had more plaques and more IAE also compared to other SSc patients (after adjustment for age, gender and disease duration).

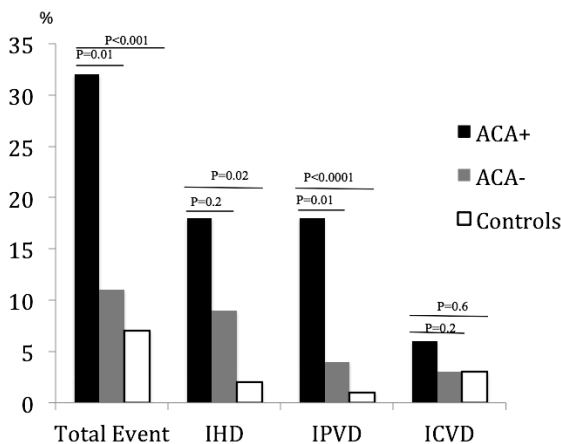


Figure 4: Ischemic arterial events in ACA+, ACA- and controls, adjusted for age, gender and disease duration

ACA+ and ACA – patients did not differ regarding inflammatory or endothelial biomarkers or traditional risk factors

Patients with SSc are at enhanced risk for IHD and IPVD especially the ACA+ SSc subgroup, which was particularly affected with both ischemic IAE and premature atherosclerosis. This group should be followed closely and modifiable cardiovascular risk factors should be treated at an early stage.

Paper II

Involvement of the heart is, together with lung manifestations, a major cause of premature mortality among SSc patients. Early detection of cardiovascular involvement is therefore important. The ECG is an inexpensive, non-invasive and commonly available method used to screen for cardiac conduction abnormalities and arrhythmias but it is sometimes overlooked in favour to more advanced instruments. We performed standard 12-lead ECGs on 110 SSc patients in Stockholm County and on 105 gender and age matched population-based controls. A subgroup underwent 24-h Holter ECGs. Associations with functional outcomes of echocardiography, disease subsets, autoantibodies, and cardiovascular risk factors were investigated.

We found resting ECG-abnormalities in 31 patients and 50 % of these were due to conduction abnormalities. The most common conduction defect was left bundle branch block (LBBB). 18 controls had ECG-abnormalities but only 5 had conduction defects. Instead non-sinus rhythm was the most common abnormality in controls. Septal infarction pattern was only found in four patients but as previously described, exclusively in the patient group. All patients with a normal ECG had a LVEF >50. ECG abnormalities and/or conduction defects were not associated with autoantibodies, inflammation, disease subtype, organ involvement or disease duration. Patients had low Apo A1 levels and these were associated with both conduction abnormalities and prolonged QTc-time.

Patients also had more Holter ECG abnormalities than controls and the most common abnormality in all participants were ventricular extrasystoles. The patients also had a higher mean heart rate than controls. In 50% of the patients with holter ECG pathology, the resting ECG was normal.

It seems as conduction abnormalities and ventricular extra-systoles still are more frequent in patients than controls and are not associated with any disease

characteristics. Septal infarction pattern was exclusively found in the patients but was rare and not associated with any particular clinical characteristics. A standard 12-lead ECG is good at detecting conduction defects and a normal ECG ruled out severe LV dysfunction but to find potentially severe arrhythmias, Holter registrations should be performed.

Paper III

Cardiac dysfunction in SSc can involve a variety of structures of the heart. Yearly screening for PAH is recommended in SSc by international cardiology organisations. We performed an echocardiography in 110 patients and 105 controls, and we investigated how our findings related to cardiac biomarkers.

44 SSc patients and 23 control subjects had an abnormal echocardiogram (P=0.002). As a group SSc patients had lower (but normal) left ventricular ejection fraction (LVEF, P=0.02), more regional hypokinesia (P=0.02) and more valve regurgitations (p=0.01) than controls. 13 patients and 4 controls had previous IHD. SSc patients had low right ventricular (RV) function, mostly in combination with LV abnormalities/dysfunction. Fifteen patients versus none of the controls had an elevated ePAP. NTproBNP and hs-cTnI were higher in SSc patients, and both biomarkers were associated with presence of echocardiographic abnormalities.

Table 5. Biomarkers of cardiac stress and its relation to echocardiographic abnormalities:

Biomarkers	Abnormal echocardiography N: 44 Odd Ratio (CI)	Estimated PAP >34 mmHg N: 15 Odd Ratio (CI)	Valvular insufficiency N: 9 Odd Ratio (CI)	LVEF<50% and/or LV hypokinesia N: 12 Odd Ratio (CI)
NT-proBNP (ng/L)	2.5 (1.5-4.6)***	1.9 (1.2-3.2)**	2.4 (1.4-5.0)**	3.2 (1.8-7.1)****
hs-cTnI (ng/L)	2.8 (1.3-6.4)**	3.2 (1.4-8.0)**	3.2 (1.1-10.6)*	3.8 (1.7-10.1)**
Uric acid (µmol/L)	1.01 (1.00-1.01)*	1.00 (0.99-1.00)	1.00 (0.99-1.01)	1.0 (0.99-1.00)

*, p<0.05, **, p<0.01, ***p<0.001, ****p<0.0001

SSc patients have a higher prevalence of abnormal echocardiograms than controls. Low RV-function and signs of pulmonary hypertension were uniquely found in the SSc group. More SSc patients than controls had valvular insufficiencies. Both NTproBNP and hs-cTnI were associated with echocardiographic abnormalities. Our results suggest that, hs-cTnI, in addition to NTproBNP, is a useful new biomarker for cardiac disease in SSc.

Paper IV

Emerging evidence demonstrate that patients with systemic sclerosis (SSc) suffer from both micro- and macrovascular disease. Structural changes in nailfold capillaries, is one type of microvascular involvement, which has been associated with clinical microvascular symptoms such as digital ulcers and PAH. In this study we investigated the association between nailfold capillary changes and macrovascular IAE by examining 163 consecutive patients, fulfilling the new 2013 EULAR/ACR classification criteria for SSc with widefield nailfold capillaroscopy. Disease characteristics, autoantibodies, previous digital ulcers, IAE, the duration and severity of Raynaud's phenomenon (RP) and the echocardiographic/Doppler estimated pulmonary arterial pressure (ePAP) were tabulated.

Mean age was 61 ± 13 years and 83% were female. Enlarged capillary loops and avascular regions were found in 52% and 54% respectively. Mean nailfold capillary density was 13.5 ± 4.5 loops/3mm. 13% had a history of IAE, 17% had an ePAP>35 and 39% had a history of digital ulcers. Enlarged capillary loops were associated with a shorter disease duration and presence of anticentromere antibodies (ACA). Occurrence of avascular regions was associated with longer disease- and RP duration, ACA, digital ulcer and limited cutaneous SSc. A low capillary density was more common among patients with a higher ePAP, ACA, digital ulcers and IAE.

Patients with IAE had a lower nailfold capillary density, but they did not differ from other SSc patients with regard to history of digital ulcers, ePAP>35 mmHg, enlarged capillary loops or avascular regions. Our results also confirm that a low capillary nailfold density is associated with a higher ePAP and with digital ulcers.

7 DISCUSSION:

Our studies have highlighted the importance of evaluating both the micro- and macrovascular disease in SSc. I would in particular like to focus the concluding discussion on the following observations and subjects.

Subgrouping of the patients:

We found that patients with ACA had more vasculopathy than patients with other autoantibodies. The classical LeRoy subgrouping of SSc patients is defined by the distribution of skin involvement (4). It did not correlate with the frequency of vascular disease or atherosclerosis in our study. Since the new classification criteria also include patients without any skin involvement, future studies will probably use antibody subgrouping. These are easier to apply and the antibodies also seem to be more reliable, and more clearly associated with clinical manifestations. In a large study based on the EULAR database, antibody profile was superior to lcSSc/dcSSc to define and predict future organ manifestations (52). The antibodies are also stable over time and don't switch from one specificity to another (47). In some cases though, ATA can disappear after treatment, but this is uncommon.

The skin score on the other hand is variable during the course of the disease and even without any treatment, skin score declines after many years of SSc. If the patient is not diagnosed during the first years of the disease the skin thickness and the distribution of skin involvement may regress so that after some time a dcSSc patient can "look like" a lcSSc patient. For example: in our cohort, about 50% of the patients had a disease duration of more than 10 years and even if 20% of these patients considered to be dcSSc at disease onset, only 2 % still had the skin distribution characteristic of the dcSSc subtype at the time of the inclusion into this study.

Characteristics of patients and controls:

In our studies we chose to include all patients, with no exclusion criteria, to determine if macrovascular events and atherosclerosis were more frequent in SSc patients than in controls. We selected population-based controls with no other exclusion criteria than a diagnosis of SSc. By this approach we aimed to study true differences between patients and controls and also to find differences with regard to traditional cardiovascular risk factors.

When comparing our results with others we note a difference in both the selection of patients and controls. Many studies use other patients from other

studies (see table 4) or health staff as comparators. Even if the aim is to match for age, in many of the previous studies there is often a difference in 2-5 years between patients and controls. The frequency of plaques and the IMT rise quickly in the age spans where SSc is common, especially after 60 years of age. In our study we were able to match our patients with controls very tightly, but despite pacemakers and echocardiographic abnormalities were more common among controls than expected.

When asking persons in the general population to participate as controls in a study like this it is possible that those, who feel a need for medical check-up. Possibly because of a positive CVD heredity or a previous known cardiovascular disease, are more likely to accept participation. Our study nevertheless demonstrated that SSc patients had more CVD than controls, even if the population-based controls may not have been as healthy as expected.

Evaluation of cardiac involvement:

International heart associations recommend yearly echocardiographic screening for PH in SSc patients. In clinical practice this can be difficult to pursue, especially when the aim today is to find patients with early SSc, who can have their disease for many decades before PH manifests. It has been shown in previous studies that clinical symptoms of PH are discrete and when present difficult to discriminate from symptoms of interstitial lung disease or cardiac ischemia (243). Several multicentre studies conducted the last ten years have resulted in attempts to combine clinical findings with various biomarkers or tests in order to detect PH before it is clinically overt (244-246).

In study II and III we described an enhanced frequency of both rhythm disorders and clinically significant echocardiographic abnormalities among SSc patients. Conduction disorders were common and in some cases so severe that they had caused implantation of a pacemaker. Frequent ventricular extrasystoles were also detected. In addition to higher ePAP the echocardiographic investigation also demonstrated valvular insufficiencies, hypokinesia and low LVEF among SSc patients.

Remembering that cardiac disease together with pulmonary fibrosis and PAH constitute the major causes of death in SSc it is important to routinely check for cardiac involvement. A standard ECG seems insufficient to detect cardiac dysrhythmias; Holter ECG should be conducted at least once and thereafter regularly if aberrations are detected or the patient has symptoms.

Our studies highlight the importance of a routine check-up with cardiac function test, such as echocardiography, not only for detection of suspected PH, but also for signs of systolic or diastolic dysfunction and valvular defects.

Biomarkers of cardiac damage:

NT-proBNP now belongs to the arsenal of “clinical routine” biomarkers. It is commonly used to diagnose and monitor heart function, especially in heart failure, but it is also used in the evaluation of disease severity of PH/PAH. Even if it is a good marker for cardiac failure, it can’t detect the pathophysiologic background of the heart disease. In our study III we found that cTnI was higher in patients with SSc than controls and that it was associated with echocardiographic abnormalities. The high levels of cTnI were not associated with any specific echocardiographic aberration; it seemed to be higher in both patients with elevated ePAP, valve regurgitations and reduced LVEF/hypokinesia. In some patients they were elevated in the absence echocardiographic abnormalities. There could be several explanation for the elevated cTnI seen in SSc patients:

- Underlying heart disease in SSc patients such as PAH or cardiac failure.
- Previous myocardial damage such as myocardial inflammation or ischemic heart disease.
- Cytotoxic drugs such as cyclophosphamide are known to be toxic for the heart. In oncology, cTn are used to monitor cardiotoxic effects. Interestingly in the study by Pieroni et al, 7 SSc patients with elevated cTnT had evidence of myocarditis on biopsy and the majority had an reduction of cTnI after treatment with cyclophosphamide
- Other diseases infiltrating the heart, e.g amyloidosis and sarcoidosis have elevated troponins.

Our results imply that cTn is a new potential biomarker for the surveillance of heart involvement in SSc. It is however important to study cTn further in order to validate their role as possible biomarkers for SSc related cardiac disease. It is in particular essential to determine if they can discriminate between different types of cardiac involvement, if they have any prognostic implications and if treatment can influence the levels.

Microvascular versus macrovascular disease:

Myocardial infarction, angina pectoris, stroke and peripheral arterial vascular diseases are described as typical macrovascular diseases. Our study highlights the close association between macro- and microvascular diseases.

We found a higher frequency of both IHD and IPVD in SSc patients than controls and in SSc patients; especially IPVD was associated with a reduced capillary density. In ACA positive patients we also noticed that atherosclerosis is accelerated. These results are really intriguing. It has been reported in two studies that patients who are positive for ACA are more prone to have severe digital ulcers, which may lead to amputation.(247). Despite this fact most of the recent studies today favours that an increased risk of digital ulcers is present in SSc patients with ATA.

Our results imply that the seldom-recognized macrovascular disease in the ACA positive group may cause or be an important contributor to digital ulcers.

8 CONCLUDING REMARKS AND FUTURE PERSPECTIVES:

This thesis has focused on macro- and microvascular disease in systemic sclerosis.

- Manifest ischemic arterial disease, engaging the heart and the peripheral vasculature is more common in SSc patients as compared to the general population.
- The enhanced occurrence of ischemic vascular disease in SSc is essentially confined to the ACA + SSc subgroup, and this subgroup is also affected with accelerated atherosclerosis.
- Conduction defects and arrhythmias are still as prevalent in SSc patients as they were 25 years ago.
- Patients with SSc have elevated markers of cardiac damage, and this is associated with echocardiographic findings.
- Patients with manifest ischemic arterial disease, especially peripheral arterial disease, have an impaired microcirculation.

Future perspectives

All studies in this thesis are cross-sectional. This design has the limitation that we can only study survivors of CVD in SSc. We can thus not give truly reliable answers to what are the underlying causes for the high frequency of ischemic arterial events in SSc. For this purpose it is important to follow the patients longitudinally to see who will develop future macrovascular events. We are presently following our cohort prospectively for five years with collection of clinical data and blood samples. We hope that these ongoing studies will shed more light upon the underlying factors for IAE in patients with SSc. We are also going to compare SSc patients with matched SLE patients, a patient group known to have an exceptionally high risk for CVD. The objective of this study is to find differences and similarities between these two autoimmune diseases with regard to CVD risk factors and outcomes.

We also plan to study the impact of microparticles, platelets, type 1 interferon, HLA-genotypes, phospholipid antibodies on both micro- and macrovascular disease in SSc:

9 ACKNOWLEDGEMENTS

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